NATIONAL TOXICOLOGY PROGRAM
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No. 412

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT (CAS NO. 7336-20-1) IN F344/N RATS AND B6C3F$_1$ MICE (FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

(CAS NO. 7336-20-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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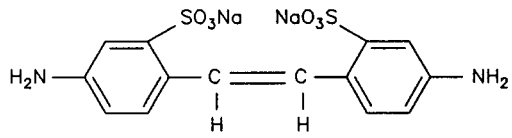
CONTENTS

ABSTRACT



4,4′-DIAMINO-2,2′-STILBENEDISULFONIC ACID, DISODIUM SALT

CAS No. 7336-20-1

Chemical Formula: $C_{14}H_{12}N_2O_6S_2 \cdot 2Na$ Molecular Weight: 414.42

Synonyms: Amsonic acid; diaminostilbene disulphonate (DASD); 2,2′-(1,2-ethenediyl)bis[5-amino-benzenesulfonic acid]; 2,2′-disulfo-4,4′-stilbenediamine; 2,2′-stilbenedisulfonic acid; 4,4′-diamino-2,2′-benzenesulfonic acid; 2,2′-(1,2-ethenediyl)bis(5-amino-) diaminostilbenedisulfonic acid; flavonic acid; *p,p′*-diaminostilbene-*o,o′*-disulfonic acid; 4,4′-diaminostilbene-2,2′-disulfonic acid

4,4′-Diamino-2,2′-stilbenedisulfonic acid, disodium salt, is used in the synthesis of dyes and optical brighteners or fluorescent whitening agents. Toxicology and carcinogenesis studies were conducted by administering the chemical (approximately 14% water, 6% sodium chloride, 4% impurities, and 76% 4,4′-diamino-2,2′-stilbenedisulfonic acid) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and Chinese hamster ovary cells.

14-Day Studies: Groups of five rats and five mice of each sex were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 14 days. All rats and mice survived to the end of the studies. The mean body weight gain of male rats receiving 50,000 or 100,000 ppm and of female rats and male and female mice receiving 100,000 ppm was significantly lower than those of the respective controls. Clinical findings included diarrhea in the rats and mice receiving 100,000 ppm. There were no chemical-related changes in absolute or relative organ weights in rats or mice. There were no gross or microscopic lesions related to chemical administration in rats or mice.

13-Week Studies: Groups of 10 rats and 10 mice of each sex were given 0, 6,250, 12,500, 25,000, 50,000, or 100,000 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 13 weeks. One female rat, six male mice, and one female mouse in the 100,000 ppm dose groups died during the studies. Mean body weight gain was significantly decreased in male rats and female mice receiving 50,000 or 100,000 ppm, in male mice receiving 25,000, 50,000, or 100,000 ppm, and in female rats receiving 100,000 ppm. Clinical findings in rats that received 50,000 or 100,000 ppm and in mice that received 100,000 ppm included diarrhea, emaciation, and hyperemia of the perineum. There were no biologically significant changes in absolute or relative organ weights or clinical pathology results in rats or mice. Histopathologic lesions present in rats receiving 100,000 ppm were bone marrow hypercellularity and chronic inflammation of the anus and rectum. Ulcerative inflammation of the anus and rectum was observed in mice receiving 25,000 ppm and above. Female mice in the 6,250, 12,500,

25,000, and 50,000 ppm dose groups had increased incidences of cystic endometrial hyperplasia.

2-Year Studies: Doses selected for the 2-year studies were based on mortality, decreased body weight gains, and the presence of diarrhea and chronic inflammation of the anus/rectum in rats and mice during the 13-week studies. Groups of 60 rats of each sex were given 0, 12,500 or 25,000 ppm and groups of 60 mice of each sex were given 0, 6,250, or 12,500 ppm 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for up to 103 weeks. Interim evaluations were performed on 10 rats and 10 mice from each dose group at 15 months. There were no biologically significant absolute or relative organ weight, clinical pathology, or histopathology findings in rats or mice administered 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in feed for 15 months.

Body Weight, Feed Consumption, Survival, and Clinical Findings in the 2-Year Studies: Mean body weights were marginally decreased for high-dose male and female rats and female mice. Feed consumption by dosed rats and mice was similar to feed consumption by the controls throughout the studies. Survival was similar among control and treated groups of rats and mice. No clinical findings related to chemical administration were observed in rats or mice.

Nonneoplastic and Neoplastic Effects in the 2-Year Studies: There were no chemical-related increased incidences of neoplasms at any site in rats. Ulcers of the forestomach or glandular stomach occurred in dosed rats (males: 1/50, 5/50, 4/50; females: 0/50, 1/50, 4/50), and may have been related to the administration of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt. There were no chemical-related incidences of neoplasms, nonneoplastic lesions, or other toxic effects in mice in the 2-year studies. Although the animals might have been able to tolerate slightly higher doses, results of the 13-week studies indicate that a doubling of the highest doses could not have been tolerated.

Genetic Toxicology: 4,4′-Diamino-2,2′-stilbenedisulfonic acid was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 with or without S9 metabolic activation. 4,4′-Diamino-2,2′-stilbenedisulfonic acid did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9.

Conclusions: Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats receiving 12,500 or 25,000 ppm. There was *no evidence of carcinogenic activity* of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female B6C3F$_1$ mice receiving 6,250 or 12,500 ppm.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Report Review Subcommittee comments and public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 4,4′-Diamino-2,2′-stilbenedisulfonic Acid, Disodium Salt

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 12,500, or 25,000 ppm in feed	0, 12,500, or 25,000 ppm in feed	0, 6,250, or 12,500 ppm in feed	0, 6,250, or 12,500 ppm in feed
Body weights			
High-dose group marginally lower than controls	High-dose group marginally lower than controls	Dosed groups similar to controls	High-dose group marginally lower than controls
2-Year survival rates			
22/50, 20/50, 24/50	30/50, 33/50, 33/50	43/50, 40/49, 42/50	43/50, 43/50, 38/49
Nonneoplastic effects			
None	None	None	None
Neoplastic effects			
None	None	None	None
Level of evidence of carcinogenic activity			
No evidence	No evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA100, TA1535, TA1537, and TA98		
Sister chromatid exchanges			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		
Chromosomal aberrations			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Report Review Subcommittee who evaluated the draft NTP Technical Report on 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, on July 9, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On July 9, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Hailey, NIEHS, introduced the toxicology and carcinogenesis studies of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt (amsonic acid), by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on the survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats. The proposed conclusions were *no evidence of carcinogenic activity* of 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, in male or female F344/N rats or in male or female B6C3F$_1$ mice.

Dr. Hayden, a principal reviewer, agreed with the proposed conclusions. To emphasize the lack of toxicity, especially in mice, he thought a statement might be added to the conclusion indicating there was no evidence of toxic or nonneoplastic activity in male or female mice. Dr. Hailey said such a statement would be added to the Abstract.

Dr. Zeise, the second principal reviewer, agreed in principle with the proposed conclusions. She said that it should be noted that male and female rats may have been able to tolerate higher doses. Dr. Hailey said he agreed that females could have tolerated higher doses but considered the doses in males to be adequate. Dr. Zeise commented that the summary tables provided combined incidence data for mammary tumors (adenomas, fibroadenomas, and adenocarcinomas) indicating significantly increased levels for female rats, and that this finding should be addressed in the report. Dr. Hailey said this combination would be eliminated because the morphological continuum seen with many neoplastic processes is not seen with fibroadenomas.

Mr. Beliczky, the third principal reviewer, agreed with the proposed conclusions. He thought it would be of value for NIOSH to evaluate the facility that manufactured the amsonic acid in view of sexual dysfunction reported by workers and uterotrophic effects observed during animal studies.

Dr. Hayden moved that the Technical Report on 4,4′-diamino-2,2′-stilbenedisulfonic acid, disodium salt, be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity.* Mr. Beliczky seconded the motion, which was accepted unanimously with ten votes.